# Ligand-Dependent Quenching of Tryptophan Fluorescence in Human Erythrocyte Hexose Transport Protein†

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ABSTRACT: D-Glucose transport by the 492-residue human erythrocyte hexose transport protein may involve ligand-mediated conformational/positional changes. To examine this possibility, hydrophilic quencher molecules [potassium iodide and acrylamide (ACR)] were used to monitor the quenching of the total protein intrinsic fluorescence exhibited by the six protein tryptophan (Trp) residues in the presence and absence of substrate D-glucose, and in the presence of the inhibitors maltose and cytochalasin B. Protein fluorescence was found to be quenched under various conditions, ca. 14–24% by KI and ca. 25–33% by ACR, indicating that the bulk of the Trp residue population occurs in normally inaccessible hydrophobic regions of the erythrocyte membrane. However, in the presence of D-glucose, quenching by KI and ACR decreased an average of −3.4% and −4.4%, respectively; Stern–Volmer plots displayed decreased slopes in the presence of D-glucose, confirming the relatively reduced quenching. In contrast, quenching efficiency increased in the presence of maltose (+5.9%, +3.3%), while addition of cytochalasin B had no effect on fluorescence quenching. The overall results are interpreted in terms of ligand-activated movement of an initially aqueous-located protein segment containing a Trp residue into, or toward, the cellular membrane. Relocation of this segment, in effect, opens the D-glucose channel; maltose and cytochalasin B would thus inhibit transport by mechanisms which block this positional change. Conformational and hydropathy analyses suggested that the region surrounding Trp-388 is an optimal "dynamic segment" which, in response to ligand activation, could undergo the experimentally deduced aqueous/membrane domain transfer.

Substrate-induced conformational changes are believed to constitute an essential feature of the human erythrocyte hexose transport protein mechanism (Holman & Rees, 1987; Walmsley, 1988; Appleman & Lienhard, 1985). Although a structural description of the proposed conformational change(s) is not yet available, kinetic studies have led to a model in which the protein alternates between two conformations (Baldwin & Lienhard, 1981). Accumulating evidence now suggests that D-glucose binding induces a change in secondary structure of the transporter protein (Chin et al., 1986, 1987). On the basis of a D-glucose-induced increase in $\alpha$-helical content, membrane incorporation of a protein segment originally at the membrane–water interface has been proposed (Pawagi & Deber, 1987). Similar domain transfer of an aqueous-accessible protein segment into the membrane has also been proposed during the transport cycle of $Ca^{2+}$-ATPase (Brandl et al., 1986).

The glucose transport protein contains six tryptophan residues distributed throughout its 492-residue primary sequence (Mueckler et al., 1985). Both the overall tertiary structure and the relationship of the integral membrane protein to the cellular lipid bilayer will determine which Trp residue(s) will be accessible to quenching by external (hydrophilic) fluorescence quenchers (Hill et al., 1986). Such quenching efficiency measurements, which employ small hydrophilic molecules such as potassium iodide (KI) and acrylamide (ACR), have been used successfully to study the accessibility of fluorescent groups in membrane proteins (Shinitsky & Rivnay, 1977; Eftink & Ghiron, 1981; Lakowicz, 1983; Eftink & Hagman, 1985). If at least one tryptophan residue is involved in direct substrate binding or located in a segment undergoing domain transfer and/or conformational change, alterations can be expected in transport protein fluorescence characteristics, i.e., emission wavelength, quantum yield, and/or susceptibility to quenching. By monitoring the intrinsic protein tryptophan emission, and by measuring the relative abilities of KI and acrylamide to quench protein fluorescence in the presence of various ligands, we have now obtained further evidence for apparent conformational or positional changes which occur in this protein during D-glucose translocation.

## Materials and Methods

*Transporter Protein Preparation.* The human erythrocyte hexose transporter (band "4.5") was isolated and purified from recently outdated blood as previously described (Baldwin et al., 1982; Pawagi & Deber, 1987). The activity of typical preparations was checked by cytochalasin B (CB) binding activity, assayed by equilibrium dialysis using [$^3$H]cytochalasin B as a tracer (Gorga & Lienhard, 1982). Protein concentration in the samples used for fluorescence was determined by the Lowry assay (Lowry et al., 1951) using bovine albumin as standard. The phospholipid content of preparations was assayed by phosphorus determination according to Bartlett (1959).

*Fluorescence Measurements.* Acrylamide was recrystallized from ethyl acetate. Potassium iodide was freshly prepared with $10^{-4}$ M $Na_2S_2O_3$ (to prevent formation of $I_3^-$) (Lehrer, 1971). For quenching studies, additions to the protein sample were made from quencher aqueous stock solutions (8.3 M acrylamide and 5 M KI); dilution never exceeded 10%, and in each experiment, the amount of quenching was corrected for the dilution factor. As acrylamide absorbs at 295 nm, an additional correction factor was applied as described by Parker (1968).

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Fluorescence measurements were performed on a Spex Fluorolog fluorescence spectrophotometer controlled with a Model Spex, DM 1B computer. Protein fluorescence was excited at 295 nm to minimize interference from protein tyrosine groups (Eftink & Ghiron, 1976). Protein fluorescence changes were monitored at the emission maximum (340 ± 2 nm). As the changes in intrinsic fluorescence and relative quenching parameters were small, slit widths on excitation and emission monochromators were adjusted to give optimal changes (i.e., 5 nm). Instrument time was set at 1–2 s, the value reported to be optimal for recording small changes in signals (Karlish & Yates, 1978). Fluorescence intensities reported are relative values and are not corrected for wavelength variations in detector response.

Experiments were performed at 25 °C. The glucose transport protein was typically suspended in 5 mM phosphate buffer, pH 7, containing 0.1 M sodium chloride and 0.1 mM EDTA. Protein concentration was typically 25–50 μg/mL. The suspension was sonicated briefly and was allowed to stand at room temperature for 15 min prior to use. To increase reproducibility, additions were made directly to the cell without removing it from the cell holder. A constant rate of stirring was maintained. No measureable difference in quenching by KI could be detected when experiments were performed in the absence or presence of KCl (up to a concentration of 0.15 M), indicating that the system was insensitive to ionic strength effects in this range. As further controls, spectra of a lipid mixture with similar composition as the transporter preparation (Baldwin et al., 1981), and spectra of cholesterol, were recorded under conditions identical with protein experiments, from which it was determined that neither of these components contributed significant emission through the region of interest (310–375 nm). All samples were particulate but optically clear suspensions; no anomalous changes due to light scatter were observed upon additions of ligands and/or quenchers. An apparent weak maximum near 415 nm, for which no specific excitation band could be detected in the range 285–400 nm, and which was also present in lipid mixtures of composition similar to the lipids in protein preparations, was therefore regarded as nonspecific fluorescence which did not arise from the transport protein.

*Data Analysis.* Quenching was analyzed according to the standard Stern–Volmer relationship, $F_0/F = 1 + K_a[q]$, where $F_0$ is the fluorescence in the absence of the quencher, $F$ is the fluorescence at molar quencher concentration [q], and $K_a$ is the Stern–Volmer quenching constant obtained from the slope of a plot of $F_0/F$ vs [q] (Eftink & Ghiron, 1976). While some investigators have used the modified Stern–Volmer equation originally proposed by Lehrer (1971) for such analyses, this treatment is deemed appropriate only when Trp residues can be clearly divided into two distinct populations (i.e., those "accessible" and "not accessible" to aqueous solvent), a specific assumption which could not be made in the present highly complex glucose transport protein system.

*Circular Dichroism Measurements.* Circular dichroism (CD) spectra were recorded on a Jasco J-41A spectropolarimeter, at a scanning speed of 5 nm/min, using a time constant of 4 s. Spectra shown are each the average of eight scans calculated every 1 nm.

## Results

*Intrinsic Tryptophan Fluorescence of the Transport Protein.* Trp fluorescence is sensitive to the polarity of the surrounding environment, having a maximum at 350 nm in water and at 329 nm in dioxane; in proteins where Trp residues are known to be located in a nonpolar region, maxima are in the range of 310–324 nm (Konev, 1967). The fluorescence spectrum of a typical preparation of the human glucose transport protein reconstituted in its endogenous lipids is characterized by $\lambda_{max}$ centered at 338 ± 2 nm (Figure 1), indicating that the spectrum is likely a composite of Trp residues distributed between the polar (exposed to aqueous) and nonpolar (located within the hydrophobic region of the lipid membrane) environments. When D-glucose (190 mM) is bound to the transporter, a decrease in the intrinsic Trp fluorescence of the protein is noted (Figure 1A), in agreement with earlier reports (Carruthers, 1986; Krupka, 1972). L-Glucose, which does not bind to the transporter, had no significant effect on the fluorescence spectrum (Figure 1B). Maltose, a disaccharide inhibitor of D-glucose transport (Krupka, 1972), induced a decrease in intrinsic fluorescence intensity (Figure 1C), as did D-glucose. A similar decrease in intrinsic Trp fluorescence at 338 nm was also observed in the presence of the inhibitor cytochalasin B (2.5 μM) (Figure 1D). These intrinsic fluorescence data are summarized in Table I. While ligand-induced percentage changes in fluorescence emission spectra were reproducible within each individual transporter preparation, there was some variation in the spectra above 380 nm (e.g., Figure 1C,D) among different protein preparations, an effect which may be attributed to small differences in lipid/protein ratios or cholesterol content (Baldwin et al., 1982; Pawagi & Deber, 1987) as well as to variations in the sizes of the vesicles.

*Quenching of Protein Fluorescence by Potassium Iodide.* Fluorescence quenchers have been widely used to study the relative accessibilities of fluorescent groups in membrane

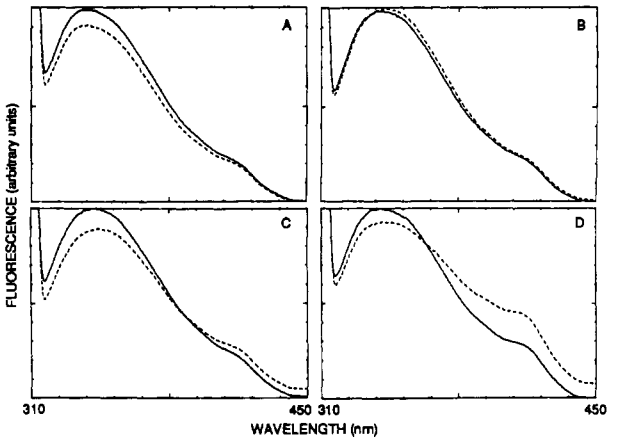


FIGURE 1: Fluorescence emission spectra of the human erythrocyte hexose transport protein in the presence of various ligands. Fluorescence was measured at 22 °C in 5 mM phosphate buffer, pH 7, 0.1 M NaCl, and 0.1 mM EDTA. Excitation wavelength, 295 nm. Slits were set at 5 nm. See Materials and Methods for further details of these experiments. The added ligands are (A) D-glucose, 190 mM, (B) L-glucose, 190 mM, (C) maltose, 40 mM, and (D) cytochalasin B, 2.5 μM. Dashed lines correspond to protein fluorescence in the presence of the indicated ligand. In each panel, the spectrum of the free protein is shown as a solid line; protein concentration: 25 μg/mL.

Table I: Changes in Intrinsic Fluorescence upon Ligand Binding to the Human Erythrocyte D-Glucose Transport Protein

| ligand | concn | $-\Delta F^a$ (%) |
|---|---|---|
| D-glucose | 190 mM | 8.9 ± 2.0 |
| L-glucose | 190 mM | 0[b] |
| maltose | 40 mM | 6.9 ± 1.2 |
| cytochalasin B | 2.5 μM | 8.7 ● 3.2 |

[a] $\Delta F$ = change in protein total fluorescence intensity at 340 nm. Protein concentration: 25 μg/mL. Values given (mean ± SD) are each the average of four determinations, calculated from typical spectra such as shown in Figure 1. [b] Values indistinguishable from buffer control spectra.

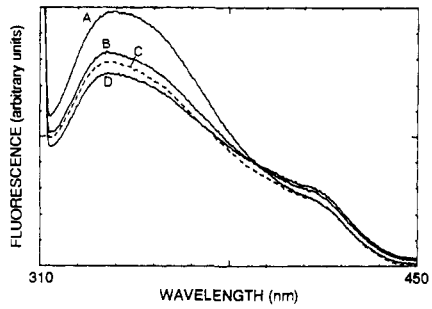


FIGURE 2: Quenching of the intrinsic Trp fluorescence of human erythrocyte hexose transport protein (30 μg/mL) by KI (200 mM). Fluorescence was measured at 22 °C in 5 mM phosphate buffer, 0.1 N NaCl, and 0.1 mM EDTA at pH 7. Excitation wavelength, 295 nm. Excitation and emission slits were set at 5 nm. Fluorescence was normalized at 336 nm to correct for intrinsic effects of additions of D-glucose and maltose (see text), so that increments shown in these two curves are due purely to quenching phenomena. The spectra are (A) free protein, (B) protein + D-glucose + KI, (C) protein + KI, and (D) protein + maltose + KI.

Table II: Ligand Dependence of Trp Fluorescence in Human Erythrocyte D-Glucose Transport Protein in the Presence of Hydrophilic Quenchers[a]

| | % decrease in protein fluorescence | | ligand-induced % change in quenching ability[b] | |
|---|---|---|---|---|
| sample | +KI | +acrylamide | +KI | +acrylamide |
| protein | 17.8 | 29.3 | | |
| +D-glucose | 14.4 | 24.9 | −3.4 ± 1.3 | −4.4 ± 1.2 |
| +maltose | 23.7 | 32.0 | +5.9 ± 3.0 | +3.3 ± 1.8 |

[a] Protein concentration: 30 μg/mL. Quencher concentration: 200 mM. Values are each the averages of three determinations, calculated from typical spectra such as shown in Figures 2 and 4. [b] Mean ± SD.

proteins (Eftink & Hagman, 1985; Shinitsky & Rivnay, 1977; Eftink & Ghiron, 1981; Lakowicz, 1983; Lehrer, 1971; Mitaku et al., 1984; Peerce & Wright, 1987; Otoda et al., 1988; Tyson & Steinberg, 1987). Quenching of the protein Trp fluorescence by KI, which is known to quench fluorescence by a collisional quenching mechanism (Lehrer, 1971), is shown in Figure 2. The extent of quenching ranged between 14 and 24% (measured at 340 nm) (Table II), indicating that greater than 75% of the total protein Trp fluorescence is not available for quenching by KI. Most significantly, these experiments demonstrated that the ability of KI to quench the protein Trp fluorescence *decreased* by an average of 3.4% in the presence of D-glucose (Figure 2B).

Eftink and Ghiron (1976) have reported that $K_a$, derived from the slope of the Stern–Volmer plot, can, in effect, be taken as a crude estimation of the accessibility of Trp residues in proteins. Stern–Volmer plots for KI quenching of transporter fluorescence in the absence and presence of D-glucose, and of the inhibitor cytochalasin B, with fluorescence emission monitored at 340 nm, are shown in Figure 3. Quenching of Trp fluorescence by KI in the presence of cytochalasin B was found to be the same as that for the free protein (not shown in Figure 2). Thus, the decreased slope of the plot in the presence of D-glucose (Figure 3C) further supports the decrease in KI quenching (Figure 2; Table II) when this ligand is present. As KI does not absorb between 185 and 300 nm, data are corrected only for dilution effects (see Materials and Methods).

*Quenching of Protein Fluorescence by Acrylamide.* Parallel quenching experiments were performed with acrylamide, a polar, uncharged water-soluble molecule, which can penetrate a protein matrix as a function of protein size and dynamics (Eftink & Ghiron, 1981), but which has been shown to be

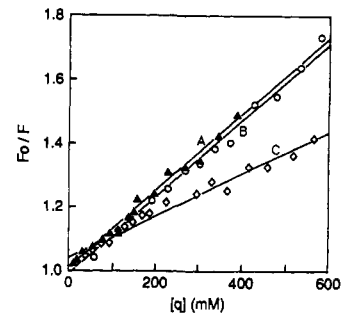


FIGURE 3: Stern–Volmer KI quenching plots of human erythrocyte hexose transporter. Protein concentration: 35 μg/mL. Fluorescence was measured at 22 °C in 5 mM phosphate buffer, pH 7. Excitation wavelength, 295 nm; emission wavelength, 340 nm. Slits were set at 5 nm. The plots are (A) free protein (▲), (B) protein + cytochalasin B (○), and (C) protein + D-glucose (◇). Values of $K_a$ calculated from the slopes of these plots, were 1.2 $M^{-1}$ for protein + KI and 0.66 $M^{-1}$ for protein + KI + D-glucose.

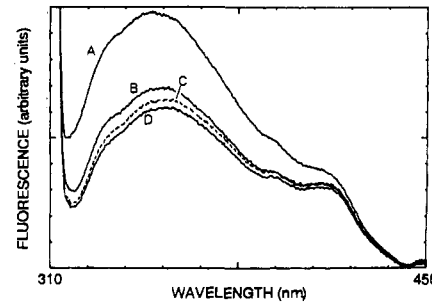


FIGURE 4: Quenching of the intrinsic Trp fluorescence of human erythrocyte hexose transport protein (30 μg/mL) by acrylamide (ACR) (200 mM). Fluorescence measured at 22 °C in 5 mM phosphate buffer, 0.1 N NaCl, and 0.1 mM EDTA at pH 7. Excitation wavelength, 295 nm. Excitation and emission slits were set at 5 nm. For additions of D-glucose and maltose, spectra were normalized at 336 nm vs the free protein spectrum, as described for the KI quenching experiments (Figure 2). The spectra are (A) free protein, (B) protein + D-glucose + ACR, (C) protein + ACR, and (D) protein + maltose + ACR.

ineffective in quenching the tryptophan fluorescence of membrane protein residues which occur in hydrophobic domains (Hill et al., 1986; Tyson & Steinberg, 1987). The fluorescence decrease induced by acrylamide in the presence of D-glucose (Figure 4B) was found to be smaller by an average of 4.4% than the quenching in the absence of D-glucose (Figure 4C), a result which, similar to KI experiments, suggests decreased accessibility of Trp residues upon addition of ligand. The quantitative changes in quenching parameters are reported in Table II.

*Quenching Experiments in the Presence of Maltose.* Bound D-glucose could act sterically to shield one or more Trp residues from collision with the quencher, and thereby account for the decrease in quenching reported above. However, results from similar experiments with the disaccharide inhibitor maltose appear to mitigate against this possibility: maltose actually *increases* Trp quenching [both with KI (Figure 2D) and with ACR (Figure 4D); data summarized in Table II], results consistent with a net increase in Trp accessibility.

*Quenching of Model Compounds.* Since ACR (200 mM) quenches protein Trp fluorescence with higher observed overall efficiency than KI, this aspect was explored further through control experiments with the model compound *N*-acetyltryptophanamide (NATA) (Carruthers, 1986). As with the protein experiments (compare Figure 2 vs Figure 4), the quenching efficiency of ACR was found to be relatively higher; thus, 80% of NATA fluorescence was quenched by ACR vs 69% by KI (data not shown). To examine the possibility that

ACR could penetrate the membrane, we measured its ability to quench the fluorescence of pyrene incorporated into dipalmitoylphosphatidylcholine (DPPC) vesicles. ACR did not quench DPPC-bound pyrene fluorescence either at 25 °C or at 45 °C (data not shown). These results are in agreement with the observation of Chalpin and Kleinfeld (1983) that ACR does not quench the fluorescence of *n*-(anthroyloxy)-fatty acid membrane probes.

## Discussion

The fluorescence quantum yield observed for the glucose transport protein is expected to reflect the mean value of the yields of all (six) Trp residues in different environments, and will be affected by interactions (dipole–dipole, dipole–ion) with surrounding protein substituents. The ligand-induced decreases observed in intrinsic fluorescence intensity (Figure 1, Table I) could result from the local protein structural changes in the ligand-bound state which alter interactions of specific Trp residues with neighboring groups. Also, nonspecific changes caused by the adsorption of ligand onto (or into) the membrane, e.g., membrane expansion and/or alteration in the orientation of lipid head groups, may also be responsible for the induced decrease in intrinsic fluorescence.

The fluorescence emission peak of the free protein did not shift significantly upon addition of ligands (Figure 1) or under various conditions of quenching by KI (Figure 2) or ACR (Figure 4). This result is consistent with previous reports of only slight shifts (1–2 nm) upon D-glucose binding (Carruthers, 1986; Krupka, 1972). Since hydrophilic quenchers are expected to influence the fluorescence of accessible, aqueous-located Trp residues preferentially, the absence of a shift in quenching experiments likely results from interresidue energy transfer. Enhanced rates of internal conversion may also be implicated.

*Conformational Consequences of Protein–Ligand Interaction.* Results from the present quenching experiments using KI and ACR, in conjunction with studies on model compounds, suggest that the bulk of the Trp residue population remains inaccessible to hydrophilic quenchers added externally to the system, and accordingly establish that these Trp's are normally located in the hydrophobic region of the erythrocyte membrane or in aqueous interior regions. However, that the percent quenching by both hydrophilic quenchers in the presence of D-glucose is lower than in its absence (Figures 2B and 4B) suggests that a Trp residue (or conceivably, fractional populations of several Trp's) does become relocated into a region less accessible to water upon D-glucose binding. If the ligand itself—which is competing with quencher molecules for the same binding site—is affecting the extent of quenching by directly shielding a Trp residue in this site, then the disaccharide inhibitor maltose would have been expected to decrease quenching even more than does D-glucose on the basis of both its larger size and, as an inhibitor, its greater affinity for the binding site. The $K_i$ for maltose is 14 mM (Krupka, 1972) which is lower than the $K_M$ (20–28 mM) reported for D-glucose (Naftalin & Holman, 1977; Wheeler & Whelan, 1988). Yet, with both quenchers, the addition of maltose increased the efficiency of quenching (Figures 2D and 4D; Table II) vs free protein. Thus, maltose appears to inhibit D-glucose transport by stabilizing an even more aqueous-exposed (vs free) transporter conformation. Such a specific nature of structural effects being probed by the quencher molecules is also consistent with our observation (Figure 1, Table I) that all the ligands examined (D-glucose, maltose, CB) decrease the protein's intrinsic fluorescence but all produce varying changes in quenching experiments.

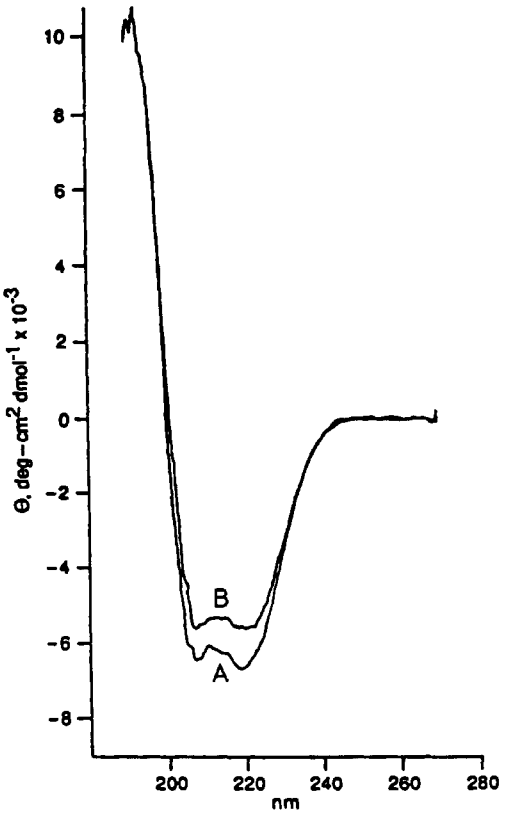


FIGURE 5: Circular dichroism spectra of purified glucose transport protein from human erythrocyte membranes (A) before and (B) after the addition of maltose (50 mM). The reconstituted protein (ca. 1 μg/mL; $2.1 \times 10^{-5}$ M) was suspended in 5 mM phosphate buffer, pH 7.4, at 22 °C. In similar experiments, addition of D-glucose induced the opposite effect [i.e., an approximately 10% *increase* in ellipticity measured at 222 nm (Pawagi & Deber, 1987)].

While selective quenching of accessible (or relocated) Trp residues is expected to produce nonlinear Stern–Volmer plots for multi-tryptophan proteins, the apparently linear plots exhibited by the glucose transporter may result from the combined effect of selective (downward curvature) and static (upward curvature) quenching (Eftink & Ghiron, 1976). The observed D-glucose-dependent decrease in the slope of the Stern–Volmer plot (Figure 3C) supports the notion that a substrate-induced locational and/or conformational change is indeed the origin of this decrease in the exposure(s) of Trp residues.

Preliminary results from circular dichroism experiments using maltose and transporter, as shown in Figure 5, are also consistent with the present quenching experiments. In contrast to an increase in ellipticity of ca. 10% (measured at 222 nm) induced by D-glucose (but no change by cytochalasin B) (Pawagi & Deber, 1987), maltose binding produces a 10% *decrease* in this helical parameter (Figure 5B). As a disaccharide consisting of two D-glucose residues, maltose has generally been suggested to bind at the substrate site but is not transported presumably for steric reasons (Carruthers, 1986). Thus, both the CD and quenching results support the suggestions of Krupka (1972) and May (1988) that maltose stabilizes a different protein conformation than does D-glucose even though both sugars apparently become bound at the same site.

*Trp-Containing Segments of the Glucose Transport Protein.* Data obtained herein concerning Trp accessibility as a function of D-glucose and maltose binding to the transporter protein, in conjunction with the availability of the protein primary sequence (Mueckler et al., 1985) and algorithms for prediction

of secondary structure (Chou & Fasman, 1978) and average hydropathy (Kyte & Doolittle, 1982) of protein segments, allow an approach toward localization of protein functional Trp residues. Considering that putative linker (aqueous-located) segments of the protein [as depicted in the model of Mueckler et al. (1985)] are of limited lengths, it appears unlikely to us that D-glucose binding induces a reordering—with an accompanying decrease in water accessibility of a Trp residue—of the secondary/tertiary structure within a large extracellular aqueous domain. From the present work, three states of the protein with respect to the membrane have been identified—free protein, a D-glucose-dependent transport active form, and a maltose-inhibited form—that are related by putative positional/domain changes of a Trp-containing segment. Thus, while we cannot specify whether the D-glucose binding site is sequentially adjacent to such a functional Trp residue, or whether substrate binding induces an allosteric event via protein tertiary structure, our model predicts the existence of a "dynamic segment" surrounding a Trp residue which can be located alternately in an aqueous or membrane domain.

Transfer of information through the conformational changes associated with such movement of a protein (or segment thereof) from aqueous to membrane domains is widely observed in biochemistry [see Deber et al. (1989) and references cited therein]. As examples, the lethal effects on cells of diphtheria toxin (Neville & Hudson, 1986; Brasseuret et al., 1986) and the bee venom peptide melittin, which can insert into membranes and lyse cells (Eisenberg, 1984), depend in part upon the penetration of a charged hydrophilic soluble protein into a lipid bilayer. In another instance, soluble proteins become membrane-associated in the biogenesis of biological membranes [the "membrane-trigger hypothesis" (Wickner, 1979)]. Penetration of membranes by biologically active peptides, including many that act through protein receptors, is also well established experimentally: several aqueous-soluble peptide hormones and neuropeptides having segments with hydrophobic character have been shown by spectroscopic methods to bind and penetrate phospholipid membranes, often with accompanying induction of secondary structure; amphiphilic effector molecules with documented membrane affinity include, e.g., the enkephalins (Deber & Behnam, 1984; Behnam & Deber, 1984), β-endorphin (Zetta & Kaptein, 1984), ACTH and dynorphin (Gysin & Schwyzer, 1984; Erne et al., 1985), and substance P (Schwyzer et al., 1986; Woolley & Deber, 1987).

To aid in developing criteria for identifying a "dynamic segment" in the glucose transport protein, we first surveyed the primary sequences of a group of extracellular cytotoxic proteins. Segments of these polypeptides [including colicin $E_1$ (Iwashita & Imahori, 1982), diphtheria toxin (Lambotte et al., 1980; Blewitt et al., 1985; Ramsay et al., 1989), δ-hemolysin (Fitton et al., 1980), and melittin (Habermann, 1972; Batenburg et al., 1987)] function analogously to the domain transfer events proposed here, i.e., they are soluble in aqueous media but undergo a lipid penetration/conformational transition in the presence of membranes. When α-helical and β-sheet conformational potentials [[$P_\alpha$] and [$P_\beta$] (Chou & Fasman, 1978)] and average hydropathy (Kyte & Doolittle, 1982) were computed from primary sequences of those segments of the cytotoxic proteins proposed (in literature cited above) to be membrane-inserted, we noted (a) absolute values of [$P_\alpha$] and [$P_\beta$] all ranged between 1.00 and 1.18, (b) [$P_\alpha$] and [$P_\beta$] were approximately equal (within 0.15) for each protein, and (c) hydropathy values ranged from 1.08 to 1.60 (data not shown). Thus, it seems feasible that membrane affinity properties of aqueous-located protein segments can be correlated with (i) conformational flexibility, i.e., those having no absolute or relative preference for either α-helix or β-sheet, and (ii) intermediate values of positive hydropathy.

Table III: Tryptophan-Containing Segments of Human Erythrocyte D-Glucose Transport Protein: Predicted Secondary Structure and Hydropathy Parameters

| segment sequence[a] | conformational potential[b] [$P_\alpha$] | conformational potential[b] [$P_\beta$] | av hydropathy[c] |
|---|---|---|---|
| $K^{38}$VIEEFYMQT$W^{48}$ | 1.08 | 1.08 | −0.84 |
| VHRYGESIL$P^{58}$ | 0.94 | 1.03 | −0.18 |
| $S^{55}$ILPTTLTTL$W^{65}$ | 0.94 | 1.16 | 1.07 |
| SLSVAIFSV$G^{75}$ | 0.98 | 1.15 | 1.93 |
| $L^{176}$DSIMGNKDL$W^{186}$ | 1.01 | 0.95 | −1.60 |
| PLLLSIIFI$P^{196}$ | 0.99 | 1.19 | 2.30 |
| $I^{353}$ALALLERLP$W^{363}$ | 1.19 | 1.05 | 1.47 |
| MSYLSIVAI$F^{373}$ | 1.07 | 1.24 | 2.06 |
| $F^{378}$FEVGPGPIP$W^{388}$ | 0.88 | 0.96 | 0.52 |
| FIVAELFSQ$G^{398}$ | 1.10 | 1.12 | 1.17 |
| $A^{406}$AIAVAGFSM$W^{416}$ | 1.10 | 1.04 | 1.40 |
| TSNFIVGMC$F^{426}$ | 0.94 | 1.19 | 1.33 |

[a] Glucose transport protein sequence as given by Mueckler et al. (1985). [b] Chou and Fasman (1978). Values for the Trp residue (not included in the calculations) are [$P_\alpha$] = 1.08 and [$P_\beta$] = 1.37. [c] Kyte and Doolittle (1982). Hydropathy value for the Trp residue (not included in the calculations) = −0.9.

When protein segments of, e.g., 10 amino acids to either side of each of the 6 glucose transporter Trp residues, are examined (Table III) by the above criteria, segments 38–58, 55–75, 176–196, and 353–373 can be eliminated from further consideration. Although of considerably higher positive hydropathy, residues 406–426 cannot be excluded as a possible candidate for the dynamic segment of the glucose transport protein. However, segment 378–398 appears to be the optimal choice. With residues 378–387 (FFEVGPGPIP) being rich in glycine and proline, this segment could have wide conformational flexibility in response to D-glucose binding; membrane-occurring proline residues have been implicated in transport protein function (Brandl & Deber, 1986; Ahl et al., 1988). On the carboxy side of Trp-388, residues 389–398 (FIVSELFSQG) have an amphiphilic character, which satisfies the above criteria best of any segment in Table III, while residues on both sides of Trp-388 also display modest positive hydropathy. This result would also be consistent with the data of Holman and Rees (1987), who have reported that Trp-388 is one of two which may be located near the ligand binding site.

## Conclusions

The present finding of a D-glucose-induced decrease in the relative quenching by the hydrophilic quenchers KI and acrylamide of Trp fluorescence of the human erythrocyte hexose transport protein is explicable by the possibility that D-glucose produces changes in the conformation/position of aqueous segment(s) of the transporter protein, which lead to a more hydrophobic environment for a Trp residue upon ligand interaction. This observation is contrasted by results using the inhibitor maltose, a disaccharide analogue of D-glucose presumed to bind the protein isosterically, which exhibited an opposite effect (i.e., relatively increased quenching). Cytochalasin B, an inhibitor whose binding site is believed to physically limit access of the D-glucose to its own binding site (Walmsley, 1988), had no significant effect on protein susceptibility to quenching, suggesting that CB binding inhibits

transport by effectively blocking the D-glucose-induced protein conformational (or locational) transition requisite for substrate translocation.

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**Registry No.** D-Glucose, 50-99-7; maltose, 69-79-4; cytochalasin B, 14930-96-2.

REFERENCES

Ahl, P. L., Stern, L. J., During, D., Mogi, T., Khorana, H. G., & Rothschild, K. J. (1988) *J. Biol. Chem. 263*, 13594–13601.

Appleman, J. R., & Lienhard, G. E. (1985) *J. Biol. Chem. 260*, 4575–4778.

Baldwin, J. M., Gorga, J. C., & Lienhard, G. E. (1981) *J. Biol. Chem. 256*, 3685–3689.

Baldwin, S. A., & Lienhard, G. E. (1981) *Trends Biochem. Sci. 6*, 208–211.

Baldwin, S. A., Baldwin, J. M., & Lienhard, G. E. (1982) *Biochemistry 21*, 3836–3842.

Bartlett, G. R. (1959) *J. Biol. Chem. 234*, 466–468.

Batenburg, A. M., Hibbeln, J. C. L., & deKruijff, B. (1987) *Biochim. Biophys. Acta 903*, 155–165.

Behnam, B. A., & Deber, C. M. (1984) *J. Biol. Chem. 259*, 14935–14940.

Blewitt, M. A., Chung, L. A., & London, E. (1985) *Biochemistry 24*, 5458–5464.

Brandl, C. J., & Deber, C. M. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 917–921.

Brandl, C., Green, N. M., Korczak, B., & MacLennan, D. H. (1986) *Cell 44*, 597–607.

Brasseur, R., Cabiaux, V., Falmagne, P., & Ruysschaert, J. (1986) *Biochem. Biophys. Res. Commun. 136*, 160–168.

Carruthers, A. (1986) *J. Biol. Chem. 261*, 11028–11037.

Chalpin, D. B., & Kleinfeld, A. M. (1983) *Biochim. Biophys. Acta 731*, 465–474.

Chin, J. J., Jung, E. K. Y., & Jung, C. Y. (1986) *J. Biol. Chem. 261*, 7101–7104.

Chin, J. J., Jung, E. K. Y., Chen, V., & Jung, C. Y. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 4113–4116.

Chou, P. Y., & Fasman, G. D. (1978) *Adv. Enzymol. Relat. Areas Mol. Biol. 47*, 45–148.

Deber, C. M., & Behnam, B. A. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 61–65.

Deber, C. M., Woolley, G. A., Deber, R. B., & Brandl, C. J. (1989) *UCLA Symp. Mol. Cell. Biol. New Ser. 86*, 97–107.

Eftink, M. R., & Ghiron, C. A. (1976) *Biochemistry 15*, 672–680.

Eftink, M. R., & Ghiron, C. A. (1981) *Anal. Biochem. 114*, 199–227.

Eftink, M. R., & Hagman, H. A. (1985) *Biophys. Chem. 22*, 173–180.

Eisenberg, D. (1984) *Annu. Rev. Biochem. 53*, 595–623.

Erne, D., Sargent, D. F., & Schwyzer, R. (1985) *Biochemistry 24*, 4261–4263.

Fitton, J. E., Dell, A., & Shaw, W. V. (1980) *FEBS Lett. 115*, 209–212.

Gorga, F. R., & Lienhard, G. E. (1982) *Biochemistry 21*, 1905–1908.

Gysin, B., & Schwyzer, R. (1984) *Biochemistry 23*, 1811–1818.

Habermann, E. (1972) *Science 177*, 314–322.

Hill, B. C., Horowitz, P. M., & Robinson, N. C. (1986) *Biochemistry 25*, 2287–2292.

Holman, G. D., & Rees, W. D. (1987) *Biochim. Biophys. Acta 897*, 395–405.

Iwashita, Y., & Imahori, K. (1982) *J. Biol. Chem. 257*, 6446–6451.

Karlish, S. J. D., & Yates, D. W. (1978) *Biochim. Biophys. Acta 527*, 115–130.

Konev, S. V. (1967) in *Fluorescence and Phosphorescence of Proteins and Nucleic Acids* (Udenfriend, S., Ed.) pp 9–59, Plenum Press, New York.

Krupka, R. M. (1972) *Biochim. Biophys. Acta 282*, 326–336.

Kyte, J., & Doolittle, R. F. (1982) *J. Mol. Biol. 157*, 105–132.

Lakowicz, J. R. (1983) in *Principles of Fluorescence Spectroscopy*, Plenum Press, New York.

Lambotte, P., Falmagne, P., Capiau, C., Zanen, J., Ruysschaerts, J., & Dirck, J. (1980) *J. Cell Biol. 87*, 837–840.

Lehrer, S. S. (1971) *Biochemistry 10*, 3254–3263.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.

May, J. M. (1988) *J. Biol. Chem. 263*, 13635–13640.

Mitaku, S., Wright, K., Best, L., & Jahnig, F. (1984) *Biochim. Biophys. Acta 776*, 247–258.

Mueckler, M., Caruso, C., Baldwin, S. A., Panico, M., Blench, I., Morris, H. R., Allard, W. J., Lienhard, G. E., & Lodish, H. F. (1985) *Science 229*, 941–945.

Naftalin, R. J., & Holman, G. D. (1977) in *Membrane Transport in Red Cells* (Ellory, J. C., & Lew, V. L., Eds.) pp 257–300, Academic Press, New York.

Neville, D. M., Jr., & Hudson, T. H. (1986) *Annu. Rev. Biochem. 55*, 195–224.

Otoda, K., Ohtoyo, T., Kimura, S., & Imanishi, Y. (1988) *Peptide Chemistry* (Shiba, T., & Sakakibara, S., Eds.) pp 725–728, Protein Research Foundation, Osaka, Japan.

Parker, C. A. (1968) in *Photoluminescence of Solutions*, pp 220–222, Elsevier/North-Holland, New York.

Pawagi, A. B., & Deber, C. M. (1987) *Biochem. Biophys. Res. Commun. 145*, 1087–1091.

Peerce, B. E., & Wright, E. M. (1987) *Biochemistry 26*, 4272–4279.

Ramsay, A., Montgomery, D., Berger, D., & Freire, E. (1989) *Biochemistry 28*, 529–533.

Schwyzer, R., Erne, D., & Rolka, K. (1986) *Helv. Chim. Acta 69*, 1789–1797.

Shinitsky, M., & Rivnay, B. (1977) *Biochemistry 16*, 982–986.

Tyson, P. A., & Steinberg, M. (1987) *J. Biol. Chem. 262*, 4644–4648.

Walmsley, A. R. (1988) *Trends Biochem. Sci. 13*, 226–231.

Wheeler, T. J., & Whelan, J. D. (1988) *Biochemistry 27*, 1441–1450.

Wickner, W. (1979) *Annu. Rev. Biochem. 48*, 23–45.

Woolley, G. A., & Deber, C. M. (1987) *Biopolymers 26*, S109–S121.

Zetta, L., & Kaptein, R. (1984) *Eur. J. Biochem. 145*, 181–186.